PATENT
Docket No. 204372000320

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on November 4, 1996.

Nancy J. Robins
NANCY J. ROBINS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

DECLARATION OF LYNN E. SPITLER PURSUANT TO 37 C.F.R § 1.132

Box AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Lynn E. Spitler, declare as follows:

1. I am a coinventor in regard to the above-referenced patent application, and have been supervising clinical trials using antitumor vaccines which contain recombinant human prostate-specific antigen (PSA) as the active ingredient. I am an experienced immunologist and medical doctor. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. I note that The Examiner makes the point several times that previous attempts to actively immunize patients with prostate adenocarcinoma cells admixed with adjuvant have shown little or no therapeutic benefit. However, the use of whole tumor cells is not analogous to the use of recombinant protein such as purified PSA. Whole PSA is not represented on the surface of the

tumor cells; thus, the patients would not be expected to be effectively immunized to PSA via this approach. PSA is synthesized within the tumor cells and secreted; therefore, the patients' immune system might be exposed to small amounts of PSA through this approach as some of the tumor cells die and release the internal PSA; these small amounts of antigen would be presented to the immune system in the context of all the other antigens present on and in the tumor cells. This would not be likely to result in an immune response to the PSA. Peptides derived from PSA are present on the surface of the tumor cells, presented in the context of HLA molecules. For these to induce an immune response, it would be expected that they would have to be taken up by the professional antigen presenting cells and represented on the surface of these cells. Again, this would be occurring in the presence of all the other antigens present on and in the tumor cells.

3. Thus, one cannot take failure of the approaches using whole tumor cells to indicate that immunization with specific antigens will fail (including antigens overrepresented in the prostate gland, an immunologically effective portion thereof, or an antiidiotypic antibody). Indeed, it is the recognition that the use of pure antigens may represent a more effective means of immunization for cancer therapy which has led to intense activity in this field and numerous clinical trials (Spitler, L.E., Engineered Vaccines for Cancer, *Sixth International Congress on Anti-Cancer Treatment* (1995) Paris, February 6-9, 1996; Spitler, L.E., Cancer Vaccines: The Interferon Analogy, *Cancer Biotherapy* (1995) 10:1-3 (copies attached).

4. Clinical trials in a number of patients have been initiated using recombinantly produced human PSA. PSA is a well known glycoprotein with a molecular weight of 33-34 kDa. PSA was cloned, expressed, and produced by large scale suspension cultures of High Five™ insecT-cells infected with recombinant PSA-baculovirus. PSA has the amino acid sequence:

D L I V G G W E C E K H S Q P W Q V L V

A S R G R A V C G G V L V H P Q W V L T

A A H C I R N K S V I L L G R H S L F H

P E D T G Q V F Q V S H S F P H P L Y D

M S L L K N R F L R P G D D S S H D L M

L L R L S E P A E L T D A V K V M D L P

T Q E P A L G T T C Y A S G W G S I E P

E E F L T P K K L Q C V D L H V I S N D

V C A Q V H P Q K V T K F M L C A G R W

T G G K S T C S G D S G G P L V C N G V

L Q G IT S W G S E Q C A L P E R P S L

Y T K V V H Y R K W I K D T I V A N P

PSA was purified from the culture supernatants by affinity chromatography using a monoclonal antibody specific to PSA and incorporated into liposomes of the following composition:

Each ml. (one dose) contained:

Component	Quantity (mg/ml)
Prostate Specific Antigen	Approximately 0.10
Monophosphoryl Lipid A	0.20
Dimyristoyl phosphatidylcholine	61.01
Dimyristoyl phosphatidylglycerol	6.89
Cholesterol	29.00
Polysorbate 80	0.10*

Buffer: 20 mM TRIS-glycine in 140 mM NaCl

*Maximum quantity that can be incorporated. The actual amount incorporated is unknown.

5. Six (6) patients were immunized with the prostate cancer vaccine described above. Each patient was given 1 ml of the vaccine intramuscularly, divided into 2 sites, on days 0, 30, and 60. An additional two (2) patients have been treated by intravenous administration of the product with the same dose and schedule of administration. All patients were carefully monitored for adverse effects through clinical and laboratory evaluation. No adverse event attributable to the vaccine was observed in any patient. Specifically, there were no adverse events suggesting an autoimmune reaction to cross-reacting antigens.

6. Immunologic tests of T and B cell responses were performed before each immunization and 2 weeks after each immunization. Evidence of T-cell immune responses was observed. (Harris, D.T., et al., Active Specific Immunization of Patients with Hormone-refractory Prostate Cancer using OncoVax-P™, ASCO Proceedings (1996)) (copy enclosed).

7. For immunologic testing of patients, a pool of peptides representing CTL epitopes of PSA was used:

Amino Acid Numbers	Sequence
29-37	VLVHPQWVL
98-106	MLLRLSEPA
141-150	FLTPKKLQCV
146-154	KLQCVDLHV
154-163	VISNDVCAQV

Peripheral blood mononuclear cells were harvested at the times indicated and incubated with the PSA peptide pool. On the third day of culture, Interleukin-2 (IL-2) was added. On day 7, the cultures were restimulated with autologous antigen presenting cells and the PSA peptide pool. The cultures were assayed on day 19 to determine the levels of gamma interferon and Interleukin-4 (IL-4) production. Results in the first four patients studied showed an increase in the production of these cytokines in some of the samples after immunization, as compared to before immunization, thus indicating a T-cell response. These results are shown in Exhibit B.

8. The foregoing results show that in clinical trials, the vaccine of the invention causes no adverse side effects sufficient to undermine its efficacy and that the vaccine is capable of eliciting an immune response to the PSA antigen mediated by T-cells.

9. In more detail, in regard to safety, there were no local reactions at the injection site, no symptoms of prostatitis, no signs of autoimmune disease, no malaise or fevers, and no signs of allergic reactions.

10. All of the patients had metastatic disease, had failed hormonal therapy, and had rising levels of PSA at the time of entry into the study.

11. As shown in the table below, and in Exhibit B, two of the six patients (patients no 2 and no. 3) had immulogical responses to PSA and three others had some suggestion of reaction (patients no. 1, no. 4 and no. 5). Lymphocytes from patient no. 2 showed proliferation to PSA and to PSA peptides as well as production of the cytokines γ-interferon and interleukin-4 in response to PSA peptides. The lymphocytes from patient no. 3 showed proliferation in response to PSA in two separate tests and this patient had a positive skin response to PSA. We were not able to measure CTLs directly because the assay is still under development; however, the cytokine production in response to PSA peptide stimulation shown in two patients is correlated with CTL development. The following table summarizes the results obtained. N.T. refers to not tested.

Immulogic Responses Summary							
Patient #	PSA Skin Test	Lympho #1 PSA	Lympho #2 PSA	Lympho Peptide	Cytokine Gamma IFN	Cytokine IL-4	Antibody
1. AW	-	-	-	+/-	-	+	0
2. JH	N.T.	+	-	+	+	+	0
3. MD	+	+	+	-	-	+/-	+/-
4. MED	-	N.T.	-	-	+/-	-	0
5. HN	-	N.T.	+/-	-	N.T.	N.T.	0
6. JLB	-	N.T.	-	-	N.T.	N.T.	0

12. Exhibit C contains copies of overhead transparencies prepared for formal presentation of results of the clinical study.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Tiburon, California on

11/1/96 Lynn E Spitler

Date Lynn E. Spitler

CURRICULUM VITAE

LYNN E. SPITLER, M.D.

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Social Security #366-38-1697

Education:

University of Michigan, Ann Arbor, Michigan		1956-1959
University of Michigan Medical School Ann Arbor, Michigan M.D. (cum laude)		1959-1963

Training:

Intern	Highland Alameda County Hospital Oakland, California	1963-1964
Resident	University of California School of Medicine San Francisco, California	1964-1966
Research Fellow	H.S. Lawrence, M.D. Department of Medicine - Immunology New York University New York, New York	1966-1967
Immunology Trainee	H. Hugh Fudenberg, M.D. University of California School of Medicine San Francisco, California	1967-1969

EXHIBIT A

Teaching Appointments and Employment:

Instructor of Medicine in Residence University of California School of Medicine San Francisco, California 94143	1970-1971
Assistant Professor of Medicine in Residence University of California School of Medicine San Francisco, California 94143	1971-present
Research Associate Cancer Research Institute University of California School of Medicine San Francisco, CA 94143	1971-1978
Director, Melanoma Center Northern California Health Center San Francisco, California 94118	1978-1990
Director of Research Children's Hospital of San Francisco San Francisco, California 94118	1978-1981
Member, Graduate Group in Comparative Pathology Department of Comparative Pathology University of California, Davis Davis, California	1976-1981
Senior Vice President XOMA Corporation 2910 Seventh Street Berkeley, California 94710	1981-1988
Associate Scientific Director Biotherapeutics, Inc. 357 Riverside Drive Franklin, Tennessee, 37065-1676	1988-1989

Director 1990-present
Northern California Melanoma Centers
1895 Mountain View Drive
Tiburon, California 94920

President 1991-present
Jenner Technologies
1895 Moountain View Drive
Tiburon, California 94920

Awards and Honors:

Recipient: Dernham Senior Fellowship 1969-1971
California Div. of the American Cancer Society

Recipient: Research Career Development Award 1971-1976
National Institutes of Health

Alpha Omega Alpha (Junior year) 1961

Outstanding Young Women of America 1968

Who's Who of American Women 1972

Who's Who in the West 1973

Board Certification:

American Board of Internal Medicine 1972
American Board of Allergy and Immunology 1974

Licensure:

Michigan	25985
New York	96454
California	C-26446

Memberships in Professional Societies:

American Association of Immunologists
American Association for the Advancement of Science
Alpha Epsilon Iota
Western Society for Clinical Research
American Federation of Clinical Research
Society of Biological Therapy
American Association for Cancer Research

Patents:

Patent #4,489,810 for "Composition and Method for Transplantation Therapy"

Patent #4,590,071 for "Human Melanoma Specific Immunotoxins"

PUBLIC SERVICE

National Review Committees:

Allergy and Immunology Research Committee, NIAID, NIH	1976-1980
Merit Review Board in Immunology, VA, Washington, D.C. (Chairman 1979-1980)	1976-1980

Editorial Boards:

The Journal of Immunology	1975-1978
The International Journal of Immunopharmacology	1979-1984
Immunologia Clinica e Sperimentale	1982-1986
Antibody Immunoconjugates and Radiopharmaceuticals (Associate Editor)	1987-present
Molecular Biotherapy	1987-present
Cancer Biotherapy	1991-present

Manuscript Reviews:

American Review of Respiratory Disease
Annals of Allergy
Annals of Internal Medicine
Archives of Dermatology
Archives of Internal Medicine
California Medicine
Cancer
Cancer Immunology and Immunopathology
Cancer Research
Cellular Immunology
Chest
Infection and Immunity
Infectious Disease and Immunology
International Journal of Immunopharmacology
Journal of Clinical Investigation
Journal of Immunology
Journal of Infectious Diseases
Journal of the American Academy of Dermatology
Molecular Biotherapy
Nature
New England Journal of Medicine
Science
The Western Journal of Medicine

Special Consultant:

National Institutes of Health Grant Reviews
National Institutes of Health Site Visits
United States Tuberculosis Panel Task Group
Atomic Energy Commission Site Visits
Public Education Panel, National Multiple Sclerosis Society
Review of Grant Applications for the National Science Foundation
Enterprise for High School Students, Medical Apprenticeship Program, San Francisco, California
Board of Directors, San Francisco Unit, American Cancer Society
Research and Human Experimentation Committee, Children's Hospital of San Francisco
U.S. Energy Research Development Administration Site Visits

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LYNN E. SPITLER, M.D.

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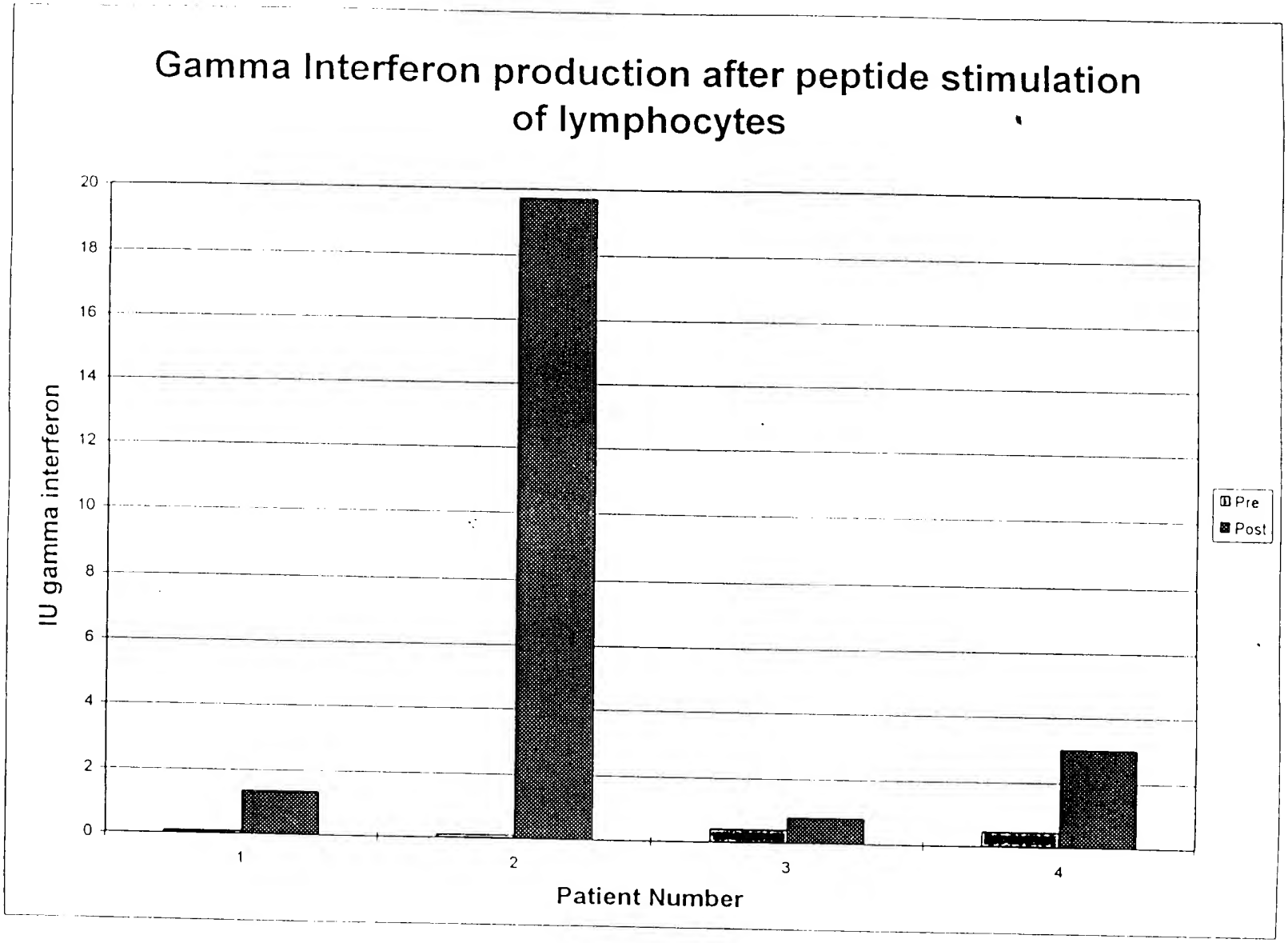
Gamma Interferon production after peptide stimulation of lymphocytes
IU gamma interferon
20
18
16
14
12
10
8
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1
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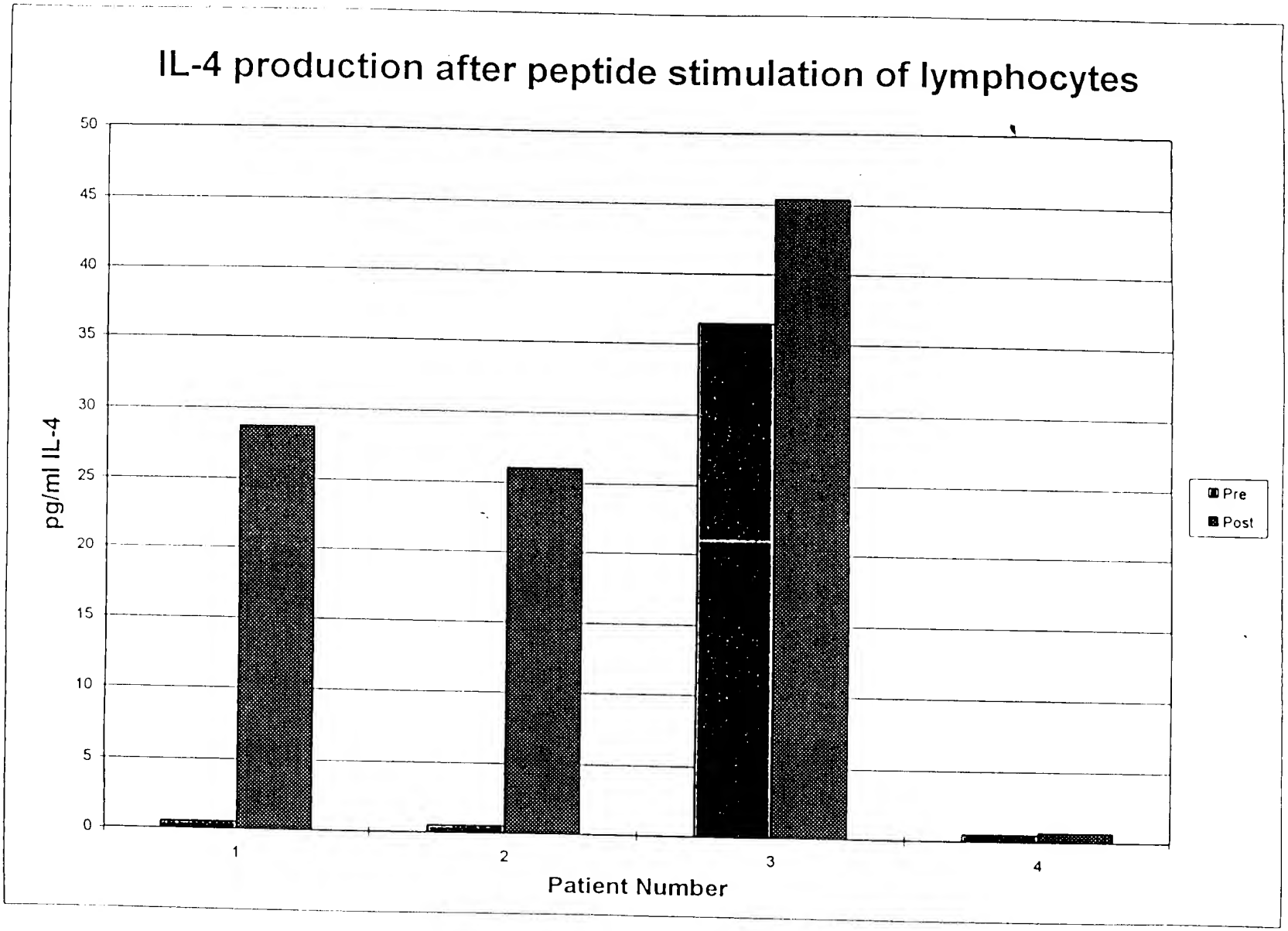
Patient Number
Pre
Post

Exhibit B


IL-4 production after peptide stimulation of lymphocytes
pg/ml IL-4
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45
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35
30
25
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10
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1
2
3
4
Patient Number
Pre
Post

Exhibit B